Key factors for causing poplar Ice Nucleation Active bacterial canker and its control techniques

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Abstract: The isolation, culture and the active determination of poplar ice nucleation active (INA) bacteria and the inoculation tests in laboratory and field were conducted, and the varieties, distribution and number of poplar INA bacteria and its pathogenicity and freezing injury property were determined. The study results showed that the INA bacteria widely spread on poplar in Northeast China and caused the frozen injury for poplar under the frost condition in Spring or Autumn, which was the key factor to induce INA bacterial canker. Through evaluation and investigation of different poplar varieties and inoculation tests, fine disease-resistant varieties and strains of poplar suitable for Northeast China were selected. Further tests for strong seedling showed that burying cuttings in sand and covering with plastic film could effectively avoid the frostbite, frozen and drought damage, reduce INA bacteria infection, and promote poplar growth. INA bacterial canker was detected early by highly specialized antiserums of INA bacteria and the agglutinated test of ring-shaped boundary surface. The inducers such as streptomycin, phenylmercuric acetae, salicylic acid and heat-killed bacteria to immerse cuttings, have obvious induced disease-resistant effect. Before poplar sprouted in early spring, through spraying the solution of frostbite agent, the control effect also was obvious.
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Introduction

Poplar INA bacterial canker is one of the most seriously forestry diseases in Northeast China. According to the investigation data, the incidence of the disease in artificial poplar forest of Northeast China is 37.6%, and the average disease index is 20.6. This disease can make tree growth decrease and stem shape become inferior; even worse, sometime it can also destroy the whole forest (Xiang *et al.* 1992).

Based on the former researches and studies in the Ninth Five-Year Plan, according to the strategic idea of keeping on developing forest in China, this paper mainly studied self-control ability of ecosystem, and the key factors for causing the disease, and at last put forward effective control techniques (Xiang *et al.* 2001a; 2001b; 2001c).

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Materials and methods

Isolation, culture and active determination of poplar ice nucleation active (INA) bacteria

Samples were taken from tumors, buds frozen to death, frozen branches and other canker spots. The isolation, culture and purification for samples were made by teasing pieces of the former materials in sterile water and streaking the resultant suspension onto meat-infusion agar or potato-dextrose agar (Cai *et al.* 1992; Zou *et al.* 2000; Zeng *et al.* 1994).

Ice nucleation active property of the purified strains of poplar bacteria was determined by "Vali" small drop freezing methods. According to their active property, they were divided into 4 types: especially strong active strains, strong active strains, middle active strains and weak active strains.

Inoculation tests of poplar INA bacteria

Inoculation tests in the laboratory and in the field were conducted in early spring, later autumn and summer. Strong INA bacteria were used to inoculate poplar wound shoots, wound stems, leaf scar and non-wound stems, at the same time, sterile water was used to stimulate contrast test.

Cross-inoculation test of poplar shoots was conducted by mixing INA bacteria with *Dothiorella gragaris* or *Coryneum populinum*, and *Dothiorella gragaris* and *Coryneum populinum* were separately inoculated as the contrast, to study the relationship between freezing injury and other induced canker disease related to INA bacteria. According to the results of the inoculation test, we analyzed the interaction among pathogen, host and environment and determined the key factors that caused the disease

Selection of fine disease-resistant varieties

Evaluation and investigations of newly selected poplar strains for timber and afforestation were made in the regional culture experimental forests of Wangkui, Jiangshanjiao and Weihei forestry bureaus of Heilongjiang Province. The evaluation, investigations and artificial inoculation experiments for regional disease-resistance were conducted in regional experimental forests of Liaodian Forestry Center of A'cheng City. Fine poplar varieties selected from North-east China were used to set up disease-resistant experimental nurseries in Wuzhan nursery of Zhaodong City and Northeast Forestry University, and evaluation, investigation and artificial inoculation experiments were conducted. In Liaoning and Jilin Province, we investigated and evaluated newly selected fine resistant forests.

Investigating method is the same as the former comment and investigation method (Liu *et al.* 1999; Wang *et al.* 1996).

Early detection technique

Four varieties of INA bacteria, such as *Pantoea agglomerans* (*E. herbicola*), *P. ananas* (*E. ananas*), *Erwinia rhapontici* and *Pseudomonas syringae* PV. syringae were used as antigens to immunize domestic rabbits respectively, and high titer products of antiserums were obtained. The test-tube precipitation tests showed that the titers for the above four varieties were 1/1024, 1/2048, 1/1024 and 1/1024 respectively, with a specific reaction rate of 95.8%. Test-tube precipitation test, glass-sheet coagulation test and agglutinated test of ring-shaped boundary surface were applied to the detection experiments.

Control test

Induced resistance test

Based on inducers selection test in the laboratory (Li 1996), streptomycin [LMS], phenylmercuric acetate [PMA], salicylic acid (SA) and heat-killed bacteria were used to conduct induced-resistance test in the field in Wuzhan nursery of Zhaodong City.

Chemical control test

The strong INA bacteria were used to inoculate Xiaohei$_{14}$ poplar before Chemical control test conducted in Wuzhan nursery of Zhaodong City, based on the drug selection tests in the laboratory, drugs which had bacteriostasis was used to spray seedlings; at the same time, contrast test was set up by spraying clear water.

Strong sprout test

In Wuzhan nursery of Zhaodong City, the cuttings of Xiaohei$_{14}$ and poplar-613 (White poplar×middle-east poplar) were stored in sand in Spring. Sand's temperatures at different levels were determined and cuttings' changes were checked when the temperature varied. Then cuttings were grown in early May and covered under plastic film, and the growth state of seedlings was observed.

Results and analysis

Key factors for causing the disease

Varieties, distribution and number of poplar INA bacteria (separation and culture test)

In northeast China, we collected samples in different seasons and commonly separated INA bacteria from tumours, diseased branches, frozen branches, buds frozen to death, black thin twig and fungi canker spots of different susceptible hosts such as Mei × Qing poplar, Beijing poplar, Cathay poplar, Xiaohei poplar, A$_{100}$ poplar and A$_{15}$ poplar. Through INA determination, we gained strong INA bacterial strains that produced ice nucleation active bacteria at –4°C, and their varieties, distribution and number were shown in Table 1.

Through identification, the main varieties are *Pantoea agglomerans* Gavim *et al.*, *Pantoea ananas* (Serrano), Mergaert *et al.*, *Erwinia rhapontici* (Millard), Burkholder, *Erwinia uredovara* (po. Et al.), Dye, and *Pseudomonas syringae* PV. *Syringae* Van Hall. They mostly spread in frozen branches and buds, swellings, canker tumours and fungi canker spots.

INA bacteria are more active and easily invasive in spring and autumn, with highest occurring frequency of 50%. *Pantoea* and *Erwinia* varieties are dominant, which exist both inside and outside diseased spot, but more exist inside.

Pathogenicity of poplar INA bacteria and characteristics of bacteria causing freeze injury (inoculation and reisolation tests)

We used strong INA strains to conduct inoculation and reisolation tests on susceptible hosts several times in spring, summer and autumn differently, then observed the characteristics of bacteria causing freeze injury and bacteria pathogenicity (Table 2-7). The results showed that the incidence by wound inoculation in spring or autumn reached 52.3%, while the incidence by non-wound inoculation or inoculation made in summer was very low or didn't cause disease at all. Cross-inoculation was also made by mixing INA bacteria with *Coryneum populinum* or *Dothiorella gragaris*; its incidence reached 26.0%. INA bacteria caused poplar freeze injury under frost condition, which is the key factor to induce INA bacterial canker. Poplar INA bacteria may strengthen invading capacity of *Coryneum*

populinum or *Dothiorella gragaris* and increase incidence of fungi canker.

Table 1. Distribution and number of poplar INA bacteria

Host	Seperation position		Seperation sample number	Bacteria		INA sample			
				Sample number	Frequency (%)	*Pantoea ewinia*	Frequency (%)	*Pseu-domonas*	Frequency (%)
A_{100} Poplar, A_{15} Poplar, Cathay poplar, Xiaoqing poplar, $Xiaohei_{14}$ poplar	Branch swellings	Surface	79	27	34.2	14	17.7	13	16.5
		Inside	91	36	39.6	26	28.6	10	11.0
A_{100} Poplar, $Xiaohei_{14}$ Poplar	Branch tumours	Surface	33	10	30.3	6	18.2	4	12.1
		Inside	42	17	40.5	15	35.7	2	4.8
MeixQing, Poplar	Stem Swellings	Surface	20	11	55.0	10	50.0	1	5
		Inside	20	14	70.0	10	50.0	4	20.0
MeixQingPoplar, A_{100} poplar, A_{15} poplar	Black thin twig		80	36	45.0	31	38.8	5	6.3
MeixQing Poplar, A_{100} poplar, A_{15} poplar	Diseased branch buds	Healthy buds	89	15	16.9	12	17.4	3	6.5
		Diseased buds	27	9	33.3	8	29.6	1	3.7

Table 2. Buds' death situation from wound inoculation of poplar shoots in spring

Strain	Inoculation number	Death number	Incidence (%)	Higher than contrast (%)
Pantoea agglomerans	50	38	76.0	30.0
Pseudomonas syringae pv. *syringae*	50	35	70.0	24.0
Pantoea anansa	50	31	62.0	16.0
Erwinia rhapontici	50	30	60.0	14.0
Sterile water	50	23	46.0	0

Table 3. Disease incidence from wound inoculation of poplar shoots and poplar stems in spring

Strain	Poplar shoots				Poplar stems			
	Inoculation number	Diseased number	Incidence (%)	Higher than contrast (%)	Inoculation number	Diseased number	Incidence (%)	Higher than contrast(%)
Pantoea agglomerans	41	32	78.0	52.3	42	24	57.1	23.8
Pseudomonas syringae pv. *syringae*	38	28	73.7	48.0	37	22	59.5	26.2
Pantoea anansa	40	26	65.0	39.3	33	27	81.2	47.9
Erwinia rhapontici	42	25	59.5	33.8	44	19	43.2	9.9
Sterile water	35	9	25.7	0	30	10	33.3	0

Table 4. Disease incidence from inoculation of poplar leaf scars and poplar stems in summer

Strain	Poplar leaf scars				Poplar stems			
	Inoculation number	Diseased number	Incidence (%)	Higher than contrast (%)	Inoculation number	Diseased number	Incidence (%)	Higher than contrast(%)
Pantoea agglomerans	50	7	14.0	1.0	48	9	18.8	3.2
Pseudomonas syringae pv. *syringae*	48	7	14.6	1.6	50	10	20.0	4.4
Pantoea anansa	45	5	11.1	-1.9	39	7	17.9	2.3
Erwinia rhapontici	40	5	12.5	-0.5	44	8	18.2	2.6
Sterile water	46	6	13.0	0	45	7	15.6	0

Table 5. Disease incidence from wound inoculation of poplar shoots and poplar stems in autumn

Strain	Poplar shoots				Poplar stems			
	Inoculation number	Diseased number	Incidence (%)	Higher than contrast (%)	Inoculation number	Diseased number	Incidence (%)	Higher than contrast(%)
Pantoea agglomerans	50	37	74.0	37.0	48	35	72.9	40.5
Pseudomonas syringae pv. *syringae*	48	34	70.8	33.8	39	31	79.5	47.1
Pantoea anansa	44	28	63.6	26.6	41	29	70.7	38.3
Erwinia rhapontici	50	29	58.0	21.0	44	27	61.4	29.0
Sterile water	46	17	37.0	0	37	12	32.4	0

Table 6. Disease incidence from non-wound inoculation of poplar stems in autumn

Strain.	Inoculation number	Diseased number	Incidence (%)	Higher than contrast (%)
Pantoea agglomerans	42	6	14.3	4.5
Pseudomonas syringae pv. *syringae*	48	5	10.4	0.6
Pantoea anansa	50	3	6.0	-3.8
Erwinia rhapontici	38	4	10.5	0.7
Sterile water	41	4	9.8	0

Table 7. Disease incidence from cross-inoculation of poplar shoots by mixing INA bacteria with *Coryneum populinum*

Strain	Inoculation number	Diseased number	Incidence (%)	Higher than contrast (%)
Mixing INA bacteria with *Coryneum populinum*	50	34	68.0	26.0
*Coryneum populinum*only (Contrast)	50	21	42.0	0

Control technique

Selection of fine disease-resistant poplar varieties

613 (White poplar × Middle East poplar), Meihei$_3$ (American poplar × European black poplar) and Heixiao$_2$ (European black poplar × Simon poplar) are selected as the fine disease-resistant varieties for Heilongjiang Province. Their disease resistance, frostbite resistance, insect resistance and volume are all obviously higher than those of formerly Xiaohei$_{14}$ poplar. The disease-resistant order is: 613 poplar> Meihei$_3$ poplar>Heixiao$_2$ poplar>Xiaohei poplar. The frostbite-resistant order is Heixiao$_2$ poplar>Meihei$_3$ poplar>613 poplar>Xiaohei poplar. The insect-resistant order is Heixiao$_2$ poplar>Meihei$_3$ poplar>Xiaohei poplar> 613 poplar. The volume order is: Heixiao$_2$ poplar >613 poplar>Meihei$_3$ poplar>Xiaohei poplar (Table 8-12).

Table 8. Investigation results in disease-resistant experimental nursery in A'cheng city

Poplar variety	Average tree height/m	Average chest diameter/cm	Average volume of single tree/m^3	Disease index of bacterial canker	Disease index of bark necrosis	Insect pest index	Freeze injury index
613	8.00	6.27	0.011326	1.36	0	8.64	0
A_{100}	7.30	5.90	0.010050	4.95	3.71	11.14	0
A_{15}	7.45	5.98	0.010676	8.82	3.68	8.09	0
A_{100}(Daqing city)	7.25	5.85	0.009865	9.72	4.02	5.56	0
Xiaohei	7.0	5.30	0.007910	10.17	11.04	7.63	0

Table 9. Investigation results of artificial inoculation experiment in disease-resistant experimental nursery in A'cheng city

Poplar variety	Bacteria variety	Inoculation number	Diseased number	Disease incidence (%)
613	*Pantoea agglomerans*	15	0	0
A_{15}	*Pantoea agglomerans*	15	7	46.7
A_{100} (Daqing city)	*Pantoea agglomerans*	15	13	86.7
A_{100} (A'cheng city)	*Pantoea agglomerans*	15	14	93.3

Table 10. Investigation results of regional culture experimental forest in region of Jiangshanjiao

Poplar variety	Average tree height/m	Average chest diameter/cm	Average volume of one tree/m^3	Disease index of bacterial canker	Insect pest index	Freeze injury index
Heixiao $_2$	16.32	14.46	0.11520	0.21	0.07	0.08
Meihei $_3$	10.84	10.63	0.04552	0.11	0.10	0.13
Xiaoqinghei×korean poplar	10.30	11.20	0.04548	0	0	1.12
Daqing poplar	9.69	11.45	0.04494	0	0	0
Xiao×Hei (Contrast)	9.66	9.93	0.03369	0.37	0.32	0.48
Tian×Xiaohei (Contrast)	8.38	8.04	0.01922	0.26	0.44	0.36

Table 11. Synthetical results of Heixiao$_2$ poplar regional culture experimental forest in Hongwei Forestry Center of Weihe Forestry Bureau

Poplar strain	Average volume of one tree/m^3	Poplar strain	Disease index of bacterial canker	Poplar strain	Freeze injury index	Poplar strain	Insect pest index
Yingchun $_5$	0.005 16	Daqing	0	Daqing	0	Heixiao $_2$	0.25
Yingchun $_1$	0.004 36	6502	0.50	A_{100}	0.25	A_{100}	0.50
Heixiao $_2$	0.004 20	B_6	0.75	6502	0.25	Xiaohei $_{14}$	1.50
A_{100}	0.003 49	Heixiao 2	1.50	Yingchun $_5$	0.50	Yingchun $_5$	2.00
Daqing	0.002 87	Xiaohei 14	4.00	Heixiao $_2$	0.75	Daqing	3.00
Xiaohei $_{14}$	0.002 57	Yingchun 5	4.50	Xiaohei $_{14}$	1.02	Yingchun $_1$	3.50
6502	0.002 12	A_{43}	6.25	A_{43}	1.75	B_6	5.00
A_{15}	0.001 94	Yingchun 1	8.00	B_6	2.25	A_{43}	5.50
B_6	0.001 81	A_{15}	8.20	A_{15}	2.75	6502	6.50
A_{43}	0.001 63	A_{100}	9.75	Yingchun $_1$	3.75	A_{15}	7.00

Table 12. Analysis on timber property of Meihei$_3$ poplar and Heixiao$_2$ poplar

Test item	Meihei$_3$ poplar	Heixiao $_2$ poplar	Daqing poplar (CK)
Average length of fibre (mm)	1.008	0.944	1.325
Average width of fibre (mm)	0.023	0.021	0.024
Length/width of fibre	43.80	44.95	55.21
Moisture content (natural dry) (%)	11.18	12.76	19.96
Density (natural dry) (g/cm^3)	0.372	0.398	0.378
Density (when moisture content is 15%) (g/cm^3)	0.381	0.404	0.390
Volume condensation rate (%)	4.143	4.292	2.900
Volume condensation coefficient (%)	0.371	0.336	0.450
Basic density (%)	0.309	0.359	0.360

Baicheng $_2$ poplar (Simon poplar×Lombardy poplar) and Bailin $_3$ poplar (Simon poplar× European black poplar) are selected as the fine disease-resistant varieties for Jilin Province. Their disease resistance, frostbite resistance, insect resistance, drought resistance, saline-alkali resistance and volume are all higher than those of formerly spread Baichengxiaohe poplar (Table 13).

Liaoning poplar, Liaohe poplar and Gai poplar that are the hyprids of American black poplar and European-American poplar, are selected as the fine disease-resistant varieties for Liaoning Province. Their disease resistance exceeds above 69% and volume exceeds above 100%, compared with that of formerly spread Shalan poplar and Xiaozhuan poplar (Table 14 – 15).

Early detection technique

The highly specialized antiserums of INA bacteria, by agglutinated test of ring-shaped boundary surface can detect poplar bacterial canker early; the accuracy of this method can reach 98% (Table 16).

Chemical control

Inducers such as streptomycin, phenylmercuric acetate (PMA), salicylic acid (SA) and heat-killed bacteria (*Pantoea agglomerans*) were used to immerse cuttings, and the average induced disease-resistant effect reached more than 70% (Table 17).

In early spring or late autumn, spraying the solution of streptomycin (600mg/kg) plus frostbite agent (10 times liquid), reached an average control effect of 75% (Table 18).

Forest management measures

Storing cuttings in sand and planting seedlings with plastic film can avoid frostbite, freeze and drought effectively and alleviate the INA bacterial infection. This method can prompt seedlings growth; and the average improvement rate was above 30% (Table 19-20).

Table 13. Investigation results in disease-resistant experimental nursery in Zhaodong and Harbin City

Poplar variety	Zhaodong				Harbin				Incidence of bacterial canker (%)
	Tree height/m	Ground diameter/cm	Leaf disease	Branching	Tree height/m	Ground diameter/cm	Leaf disease	Branching	
Zhongheifang	2.02	1.36			1.23	0.95			20.0
J_2	2.05	1.22			1.74	1.00			0
J_1	2.26	1.44			1.30	0.72			0
J_4	2.25	1.47			1.65	0.99			10.0
A_{43}	2.35	1.72	+V		0.80	0.56			35.0
613(Zhaodong)	1.95	1.54		+	1.25	1.14			10.0
A_{118}	2.13	1.40			1.31	1.10			16.7
A_{15}	2.06	1.42	+V		1.81	1.04			20.0
R_2	1.74	1.10			0.92	0.60			0
DL1-1	1.15	0.68			1.04	0.61			0
J_3	2.08	1.29							0
A_{100}	1.74	1.10			1.08	0.35			66.7
613(Harbin)					1.29	0.88		+	0
51					1.46	1.12			10.0
Zhongsui $_{12}$					1.62	1.18	+C	+	15.0
L_2					1.05	0.77		+	0
Xiaohei $_{14}$					1.74	1.46			43.3
DL1-2					0.68	0.40			0
Mei×Qing(CK)					1.58	1.05			78.4

Notes: V---*Venturia populina*; C---*Coryneum populinum*; +, ++, +++--- harmful degree, light, medium and heavy separately.

Table 14. Investigation results of comparing and assessing forests for newly selected fine disease-resistant poplar varieties in Liaoning Province

Poplar variety	Sites	Disease index of bacterial canker	Insect pest index	Freeze injury index	Age/a	Average diameter/cm	Average height/m	Volume /$m^3 \cdot hm^{-2}$
Liaoning Poplar	Gaizhou	0	0	0	5	13.9	12	30.2896
Liaoning Poplar	Xinmin	0	0	0	4	13.8	11	20.5087
Shalan Poplar(CK)	Gaizhou	0	40	0	4	6.4	7.4	5.5819
Liaoning Poplar	Xinmin	0	0	0	11	23.7	23	46.9511
Liaoning Poplar	Jinxian	0	0	0	9	22.9	22	138.4883
Liaoning Poplar	Jinxian	0	0	0	9	21.8	20	122.7489
Liaohe Poplar	Xinmin	0	0	0	11	20.1	21	95.8889
Gaixian Poplar	Xinmin	0	0	0	11	20.6	20	92.3604
Xiao zhuan Poplar	Jinxian	25.9	0	2.2	12	14.6	15	68.5041
Mei×Qing(CK)	Xinmin	21.80	0	10.5	19	15.7	19	66.3422

Table 15. Analysis on timber properties for Liaoning poplar, Liaohe poplar and Gaixian poplar

Poplar variety	Density /$g \cdot cm^{-3}$				Length /μm	Width /μm	Length/Width
	Naturally dry	Contrast with Shalan poplar (%)	Basic	Contrast with Shalan poplar (%)			
Liaohe poplar	0.446	118	0.358	116	826	21.99	37.60
Liaoning poplar	0.491	130	0.381	125	887	21.95	41.14
Gaixian poplar	0.522	150	0.417	135	940	21.08	48.65
Shalan poplar(CK)	0.378	100	0.309	100	994	26.15	38.01

Note: the data was provided by Chinese Academy of Forestry.

Table 16. Agglutinated test of ring-shaped boundary surface between extracted liquid of smooth cortex tissue of diseased plant and antiserums

Antiserum	Original plant number of known extracted liquid										Contrast		
	1	2	3	4	5	6	7	8	9	10	a	b	c
Pantoea ananas	+	+	+	+	+	+	+	+	+	+	-	-	-
Pseudomonas syringae	+	+	+	+	-	+	+	+	+	+	-	-	-
Pantoea agglomerans	+	+	+	+	+	+	+	+	+	+	-	-	-
Erwinia rhapontici	+	+	+	+	+	+	+	+	+	+	-	-	-
Mixture	+	+	+	+	+	+	+	+	+	+	-	-	-

Notes:"+"---means coagulation; "- " means no coagulation; a---antigen plus physiological saline; b---antigen plus normal serum; C---extracted liquid of healthy plant plus antiserum.

Through forest management and control measures such as afforesting right trees in proper sites, cultivating strong seedlings, planting elaborately, loosening the forest soil in time, weeding, thinning trees and sanitarily cutting, we can create a suitable environment for trees' growing, so as to enhance the hosts' resistance and trees' self-control ability and reduce the disease's harm effectively.

The above control techniques were used in control demonstration forests. Investigation results of standard plots showed that the incidence of the disease was controlled under 5%, the forests increment was raised by 70%, compared to the contrast forests, wood volume of young forests by 27.68 m^3/hm^2, and that of mature forests was increased by 178.95 m^3/hm^2 or 213.30 m^3/hm^2. This research achievement has been spread in Heilongjiang Province and the spreading area has reached to 21000 hm^2, which could produce direct economic benefit of 53 million yuan based on volume increment.

Table 17. Induced effects of different inducers on poplar in the field

Disposal	Concentration	Investigation number	Diseased number	Disease incidence (%)	Induced effect (%)
phenylmercuric acetate	100 mg/kg	33	1	3.0	90.0
phenylmercuric acetate	200 mg/kg	-	-	-	-
salicylic acid	10 mmol/L	80	2	2.5	91.7
salicylic acid	8 mmol/L	-	-	-	-
streptomycin	600 mg/kg	91	2	2.2	92.7
streptomycin	400 mg/kg	35	0	0	100
Heat-killed bacteria	10^9 /mL	82	5	6.1	79.7
Clear water (CK)		100	30	30	-

Table 18. Generally average medicine effects of different medicines

Medicine	Investigation number	Diseased number	Not diseased number	Disease incidence (%)	Generally average medicine effect (%)
White paint agent	90	31	59	34.44	31.11
White paint agent + Streptomycin×100*	90	15	75	16.67	66.67
White paint agent + Anilazine×100	90	17	73	20.00	62.22
frostbite protection agent×100 + Streptomycin×100	90	11	79	12.22	75.56
White paint agent×100+Urbazid×100	90	16	74	17.78	64.44
Streptomycin×100	90	13	77	14.44	71.11
Urbazid×100	90	19	71	21.11	57.78
White-paint agent×100	90	19	71	21.11	57.78
Contrast	90	45	45	50.00	62.22

Notes: Generally average medicine effect=(not diseased number after dealed with medicine-not disease number by contrast)/ diseased number by contrast; *--- Medicine solution is diluted with water (100times).

Table 19. The effect of covering plastic film on height and diameter growth of seedlings

Disposal	Seedling Age/a	Investigation number	Average seedling height /m	Variance of seedling height/m	Average ground diameter/m	Variance of ground diameter/cm
Covering with plastic film	0-1	50	1.42	0.20	0.97	0.15
	2-1	100	2.23	0.20	1.34	0.23
Contrast	0-1	50	1.06	0.25	0.76	0.16
	2-1	100	2.07	0.15	1.23	0.18

Table 20. Investigation results of height growth of seedlings for storing cuttings in sand

Variety	Position	Investigation number	Height growth /m	Variance	"U" value
$Xiaohei_{14}$	Upper layer	50	0.996	0.1088	2.61
	Lower layer	50	1.179	0.1433	
613	Upper layer	50	1.023	0.1474	2.75
	Lower layer	50	1.243	0.1928	

Conclusions

Poplar INA bacterial canker is a kind of disease that hosts play a leading role, and also a kind of ecological disease. That is to say, poplar INA bacteria have saprophytic and feeble parasitic properties, spreading in the nature widely and hiding in hosts for a long time. They can cause the freeze injure for susceptible poplar varieties and strains under frost condition, which is the key factor to induce INA bacterial canker. The infected poplars' cortices will turn grey and become necrosis, xylem hyperpra, then form tumour, and the trees can also be malformed and even be killed when condition is very serious.

Based on the key factor that caused poplar INA bacterial canker, following measures were put forward for the disease control: (1) Highly disease-resistant poplar varieties or strains should be selected and right trees should be afforested in proper sites. (2) Inducers such as streptomycin, phenylmercuric acetate, salicylic acid and heat-kill bacteria (*Pantoea agglomerans*) should be used to immerse cuttings. By these means, the average induced disease-resistant effect can reach more than 70%; (3) The cuttings should be stored in sand and covered with plastic film. These measures are helpful to avoiding frostbite, freeze and drought effectively, reducing the INA bacterial infection, and prompting seedlings to grow; (4) In early Spring, by spraying the solution of streptomycin mixing frostbite agent, the disease can be controlled in a certain degree before poplar sprouts.

References

Bacterial classification group of Microbe research institute of Chinese scientific graduate school. 1978. Common determination method for general bacteria [M]. Beijing: Scientific Press, 135-193.

Cai Miaoying (Skerman, V.B.D. Initial author). 1992. Determination instruction of bacteria [M]. Beijing: Scientlfu Press, 129-199.

Fang Zhongda. 1997. Research methods of plant diseases [M]. Beijing: Agriculture Press, 269-275.

Liu Xuefeng, Dong Airong, Su Fengxian, *et al.* 1999. The comment and investigation on regional test of poplar fine varieties [J]. Journal of Northeast Forestry University, **27**(1):71-74.

Liang Xunsheng. 1985. Serological technique of plant diseases [M]. Beijing: Agriculture Press, 131-133.

Li Chunjiang, Xiang Chunti, Mao Dejiang, *et al.* 1996. Study on hosts' induced resistance on polar INA bacterial canker. Forest research, (Special): 184.

Translation group of Beijing bacterial determination manual (the eighth edition). 1984. (Buchanan, R.E. Gibbons, N.E. initial author) Beijing bacterial determination manual [M]. Beijing: Scientific Press, 274-486.

Wang Ping. 1996. Contrast test on growing condition and resistance of several main poplars [J]. Jilin Forestry Science and technology, **30**: 15-17.

Xiang Cunti. 1991. Research methods of forestry disease [M]. Harbin: North-east forestry university Press, 269-275.

Xiang Cunti and Shao Liping. 1992. Integrated protect and control technique of poplar disease [M]. Harbin: The press of Northeast Forestry University, 119-172.

Xiang Cunti, Song Fuqiang, Liu Jinghua, *et al.* 2001a. The dominant factors caused by INA bacterial canker [J]. Journal of Northeast Forestry University, 109-113.

Xiang Cunti, Liu Xuefeng, Dong Airong, *et al.* 2001b. Screening for the resistant improved varieties of poplar ice nucleation bacterial canker [J]. Journal of Northeast Forestry University, **29**(3): 123-127.

Xiang Cunti, Li Gongbin, Dong Airong, *et al.* 2001c. Control techniques of poplar INA bacterial canker [J]. Journal of Northeast Forestry University, **29**(3): 134-138.

Yao junhai. 1990. Serological technique use in forestry disease research [J]. Forest pest and disease, (4):36- Scientia silvae sinicae, **35**(30): 53-57.